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STUDIES ON THE PIGMENTS OF MARINE ANIMALS

VII. CAROTENOIDS IN THE SKIN AND FINS OF SOME MARINE FISHES

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With Plate XXX, 5 Table and 5 Text-figures

The skin and fins of fishes show various colours which are mainly due to melanins and carotenoids. The carotenoids of the skin and fins of fish have been investigated by many workers and the results were summarized by FOX (1953), GOODWIN (1952) and KARRER (1950). They pointed out that xanthophyll, astaxanthin and taraxanthin are predominant but the isolation of the crystalline carotenoid has not been achieved in the majority of cases.

The present study concerns carotenoids in the skin and fins of four species of

Table 1. Species, numbers and colours of the marine fishes analysed.

Species	No. of specimens for sample	Colour of skin and fine
Parabembras curtus	14	Red
Lepidotrigla microptera	10	Orange-red
Sebastes inermis	6	Dirty brown-red
Taius tumifrons	3	Yellow-pink

Table 2. Absorption maxima of carotenoids in the skin and fins of four species of marine fish (in CS_2).

Species	Free xanthophyll	Esterified xanthophyll		Carotenoid hydrocarbon
		Astacene	Yellow xanthophyll	
Parabembras curtus	**—	~510 mμ	f(II) 438, 466, 498 mμ	**—
Lepidotrigla microptera	**—	~510 mμ	f(I) 438, 465, 497 mμ	**—
Sebastes inermis	**—	~510 mμ	f(I) 438, 467, 500 mμ	*f(I) 440, 468, 500 mμ f(II) 443, 472, 498 mμ
Taius tumifrons	**—	~510 mμ	f(I) 473, 500 mμ *f(III) 439, 466, 498 mμ	f(I) 438, 466, 498 mμ

* The main zone of chromatographic adsorption.
** The amount was too minute to determine.

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marine fish having a red, brown-red and yellow-pink colour (Table 1). The results are summarized in Table 2.

The principal carotenoid of each species is found in the fraction of esterified xanthophyll, which is divided into two fractions; one of which is red and the other is yellow. The former seems to be esterified astaxanthin, as the crystalline red ketonic carotenoid which is recognized to be identical with astacene because of its properties (crystalline form, melting point, absorption maximum alone and when mixed with authentic astacene prepared from lobster) has been isolated. The latter is a yellow xanthophyll having absorption maxima at 438, 466, 498 mμ (in CS_2) which resembles sarcinaxanthin.

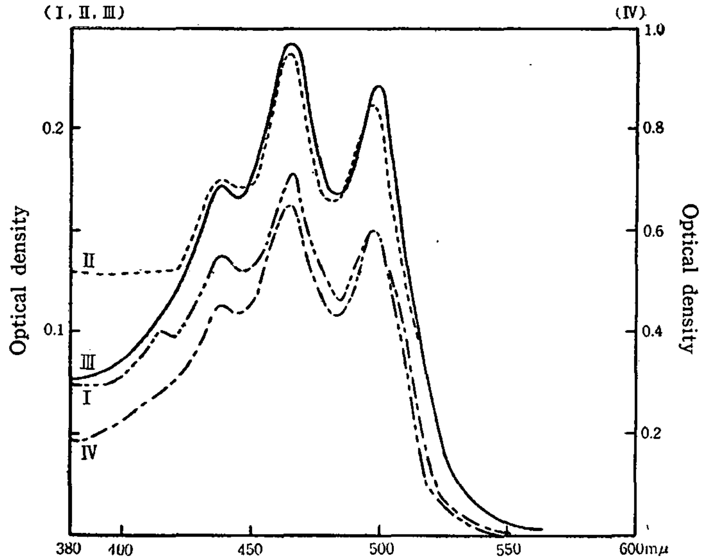

Fig. 1. Absorption spectra of the principal yellow xanthophyll separated from the skin and fins of four species of marine fish, in carbon disulphide.

I) *Parabembras curtus.* II) *Lepidotrigla microptera.*
III) *Sebastes inermis.* IV) *Taius tumifrons.*

Carotenoid	Absorption maxima in CS_2
Yellow xanthophyll from the skin and fins of marine fish	438, 466, 498 mμ
Sarcinaxanthin	436, 466.5, 499 mμ
Taraxanthin	441, 469, 501 mμ

This yellow xanthophyll is contained in each of the four species and forms a main yellow pigment component (Fig. 1).

The colour of the skin and fins of fishes examined, is affected mainly by the

concentration of astaxanthin and the yellow xanthophyll ; in the case of *Parabembras curtus* having red skin and fins, the concentration of astaxanthin is much greater than that of the yellow xanthophyll, and in the case of *Taius tumifrons* having yellow-pink skin and fins, however, the concentration of astaxanthin and yellow xanthophyll is not so different. (see Tables 4, 5 and Fig. 5)

Material and method

The specimens of the marine fishes used in this experiment are shown in Table 1. Extraction and separation of the carotenoid was carried out in the procedure shown in Table 3.

Table 3. Extraction and separation method

- Tissue
- Acetone ext.
- Petroleum ether ext.
- Wash with water
- Part with 90% methanol
  - Petroleum ether phase
    - Talc adsorption
    - Wash with petroleum ether
      - Talc (violet-red colour)
        - Acetone eluate
        - Petroleum ether ext.
        - Saponify with alcoholic alkaline solution
        - Acidify with dil. acetic acid
        - Crystal (astacene)
        - Recrystallization from hot aqeous pyridine
        - (a). Astaxanthin fraction derived from its ester
      - Filtrate (bright yellow)
        - Saponify with alcoholic alkaline solution
        - Dilute with water
          - Hypohase
          - Epiphase
            - Part with 90% methcnol
              - 90% methanol phase (joined with Hypohase)
              - Petroleum ether phase
                - Chromatographed on Al_2O_3
                - Carotenoid hydrocarbon fraction
        - Ether ext.
        - Evaporate
        - Cake
        - Dissolved in benzene
        - Chromatographed on $ZnCO_3$
        - (b). Yellow xanthophyll fraction derived from its ester
  - 90% methanol phase
    Free xanthophyll fraction

Result

The principal pigment was found in the fraction of esterified xanthophyll. Detailed results for each of marine fishes are given below.

I. *Parabembras curtus* (TEMMINCK et SCHLEGEL) (Japanese name; Ubagochi).

An amount of 60 g of skin and fins from 14 freezing specimens obtained at a fish-market was used for acetone extraction. The freshness of the samples was not so good.

Free xanthophyll fraction: None of carotenoid was detected.

Esterified xanthophyll fraction: (a) Astaxanthin fraction. Crystalline carotenoid, violet-black needle, was isolated (Plate XXX, fig. 1). It melts at 214°C and showed

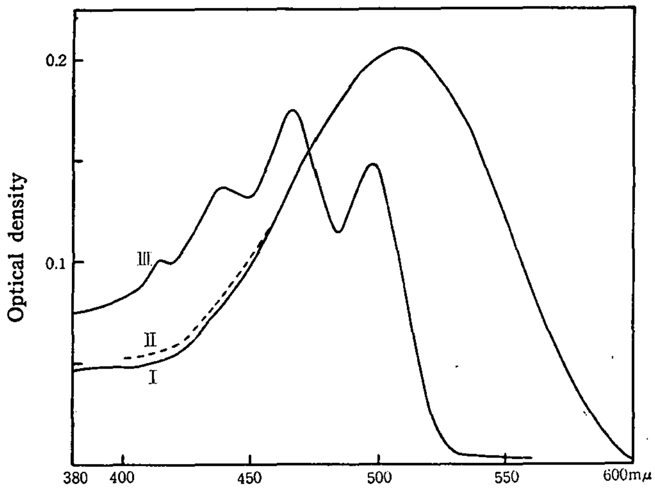

Fig. 2. Absorption spectra of carotenoids from the skin and fins of *Parabembras curtus*, in carbon disulphide.
I) Astacene from the esterified astaxanthin.
II) Mixed solution of I and lobster astacene.
III) Yellow xanthophyll zone (ii).

the absorption maximum at ~510 mμ in carbon disulphide (Fig. 2, I). CARR-PRICE reaction (+) blue, conc. H_2SO_4 reaction (+) blue. Mixed absorption maximum with astacene (isolated from a deep-water lobster, *Linuparus trigonus* VON SIEBOLD, m. p. 217°C, Plate XXX, fig. 2, absorption maximum ~510 mμ in CS_2, Fig. 3, II) was at ~510 mμ in the same solvent. (Fig. 2, II). These properties indicate that the crystalline carotenoid is identical with astacene.

(b) Yellow xanthophyll fraction. Two zones were formed when it was chromatographed on the column of zinc carbonate from benzene solution and developed with petroleum ether containing acetone (10%).

Colour of zone	Absorption maxima in CS_2
(i) Red-pink	————
(ii) Deep yellow (main zone)	413, 438, 466, 498 mμ

The absorption spectrum of (ii) was shown in Fig. 2, III.

Carotenoid hydrocarbon fraction: The colour of this fraction was too minute to determine.

II. *Lepidotrigla microptera* (GÜNTHER) (Japanese name; Kanagashira).

An amount of 70 g of skin and fins from 10 freezing specimens obtained at a fish-market was used.

Free xanthophyll fraction: The 90% methanol solution of this fraction was almost entirely colourless.

Esterified xanthophyll fraction: (a) Astaxanthin fraction. Black-violet needles, m. p. 214°C (Plate XXX, fig. 3) were isolated. Absorption maximum was at ~510 mμ in carbon disulphide. Mixed absorption maximum with astacene was at ~510 mμ in carbon disulphide. CARR-PRICE reaction (+) blue.

(b) Yellow xanthophyll fraction. Two zones were formed from the top of the column of zinc carbonate when it was developed with petroleum ether containing acetone (10%).

Colour of zone	Absorption maxima in CS_2
(i) Light pink	————
(ii) Yellow (main zone)	438, 465, 497 mμ

The absorption spectrum of (ii) was shown in Fig. 1, II.

Carotenoid hydrocarbon fraction: The amount of the carotenoid in this fraction was little.

III. *Sebastes inermis* (CUVIER et VALENCIENNES) (Japanese name; Mebaru).

An amount of 60 g of the fresh skin and fins from 6 specimens (about 15 cm in average length) collected at Okayama Prefecture was used for extraction.

Free xanthophyll fraction: The 90% methanol solution containing the pigment of this fraction was almost colourless.

Esterified xanthophyll fraction: (a) Astaxanthin fraction. Black-violet sickle-shaped needles, m. p. 221.5°C, were isolated (Plate XXX, fig. 4). The yield was about 1 mg. Absorption maximum was at ~510 mμ in carbon disulphide (Fig. 3, I). It possessed a hypophasic property and was soluble in organic solvents (chloroform, carbon disulphide, pyridine and acetone) and sparingly soluble in petroleum ether. CARR-PRICE reaction (+) violet, conc. H_2SO_4 reaction (+) blue, mixed absorption maximum with astacene showed also at ~510 mμ in carbon disulphide (Fig. 3, III).

(b) Yellow xanthophyll fraction. The bulk of the yellow pigment was found in this fraction. Bright yellow benzene solution was chromatographed on the column of zinc carbonate and developed with petroleum ether containing acetone (10%).

Deep red zone (i), moving down slowly, was found. The absorption maxima of (i) showed at 438, 467, 500 mμ in carbon disulphide (Fig. 3, IV). Any attempt to isolate the crystalline carotenoid failed.

Carotenoid hydrocarbon fraction: This faction was poor in colour. The petroleum ether solution (light yellow) was chromatographed on the column of alumina (Merck) and developed with petroleum ether containing acetone (10%).
Three zones were formed.

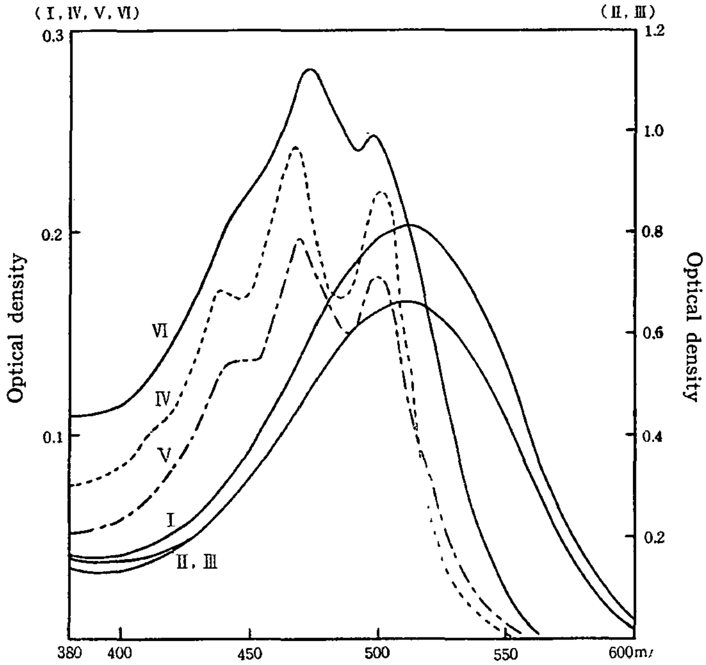

Fig. 3. Absorption spectra of carotenoids from the skin and fins of *Sebastes inermis*, in carbon disulphide.

I) Astacene from the esterified astaxanthin fraction.
II) Astacene from lobster.
III) Mixed solution of I and II.
IV) Yellow xanthophyll, zone (i).
V) Carotenoid hydrocarbon zone (i).
VI) Carotenoid hydrocarbon zone (ii).

	Colour of zone	Absorption maxima in CS_2
(i)	Bright yellow (main zone)	~440, 468, 500 mμ
(ii)	Light orange	~443, 472, 498 mμ
(iii)	Extremely light yellow	————

The absorption spectra of (i) and (ii) were shown in Fig. 3, V and VI.

IV. *Taius tumifrons* (TEMMINCK et SCHLEGEL) (Japanese name; Kidai).

An amount of 60 g of skin and fins from 3 specimens (20 cm in average length) collected at Okayama Prefecture were used.

Free xanthophyll fraction: Trace of colour was detected.

Esterified xanthophyll fraction: (a) Astaxanthin fraction. Crystalline carotenoid, m. p. 205°C, black-violet sickle-shaped needle, was isolated (Plate XXX, fig. 5). The absorption maximum was at ~510 mμ in carbon disulphide (Fig. 4, I). It had hypophasic property. CARR-PRICE reaction (+) blue, conc. H_2SO_4 reaction (+) blue. There was no displacement of the absorption maximum mixed with the lobster astacene (Fig. 4, II).

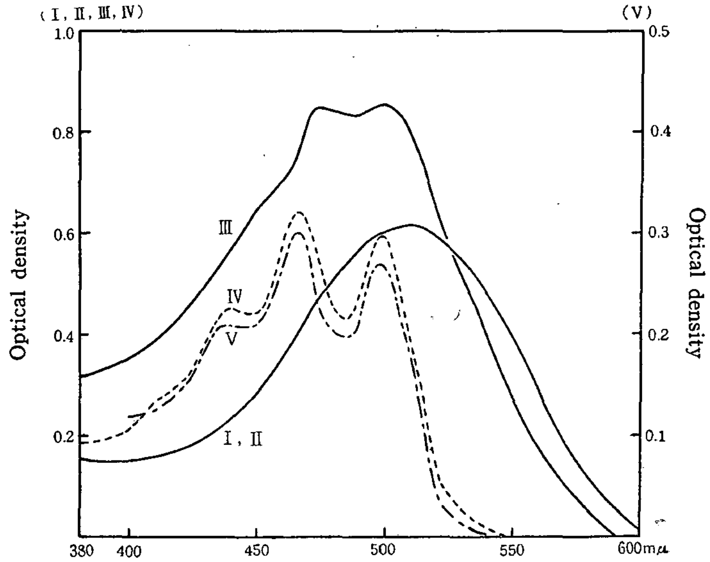

Fig. 4. Absorption spectra of carotenoids from the skin and fins of *Taius tumifrons*, in carbon disulphide.

I) Astacene from the esterified astaxanthin.
II) Maxed solution of I and lobster astacene.
III) Yellow xanthophyll zone (i).
IV) Yellow xanthophyll zone (iii).
V) Carotenoid hydrocarbon zone(i).

(b) Yellow xanthophyll fraction. The yellow colour of the specimens owed to this fraction. When the benzene solution of the pigment was chromatographed on the column of zinc carbonate and developed with petroleum ether containing acetone (10%), three zones were obtained from the top of the column.

	Colour of zone	Absorption maxima in CS_2
(i)	Bright red	473, 500 mμ
(ii)	Extremely light yellow	—
(iii)	Orange (main zone)	439, 466, 498 mμ

The absorption spectra of (i) and (iii) was shown in Fig. 4, III and IV.

Carotenoid hydrocarbon fraction: Two zones were found when the petroleum ether solution (light yellow) of this fraction was chromatographed on the column of alumina and developed with petroleum ether containing acetone (10%).

	Colour of zone	Absorption maxima in CS_2
(i)	Yellow (main zone)	438, 466, 498 mμ
(ii)	Extremely light yellow....................................	———————

The absorption spectrum of (i) was shown in Fig. 4, V.

V. Determination of concentration of the red and yellow carotenoid.

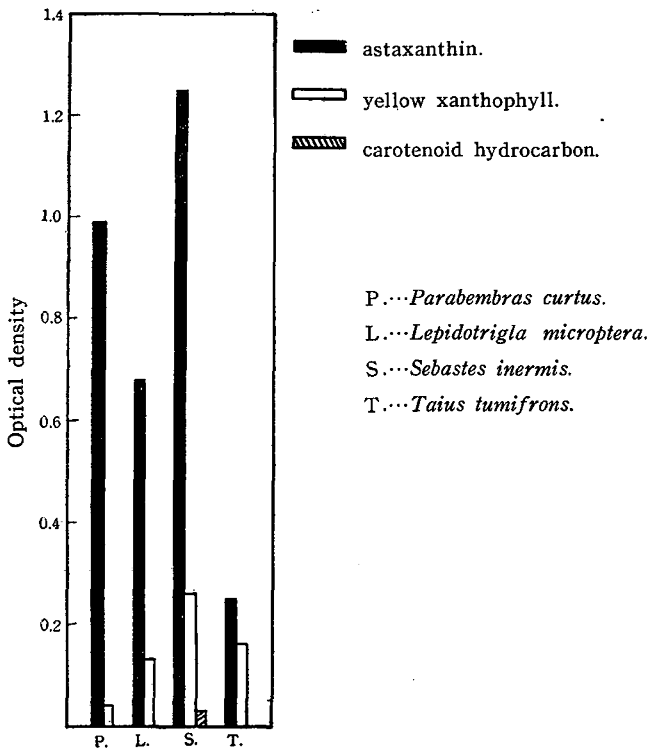

Fig. 5. Relative concentration of astaxanthin, yellow xanthophyll and carotenoid hydrocarbon in the skin and fins of four species of marine fish.

Carotenoid pigments in the 17 g of the skin and fins of each of four species of marine fish, were extracted entirely and separated into three fractions; astaxanthin (red colour), yellow xanthophyll (yellow colour) and carotenoid hydrocarbon (yellow colour). Each fraction was dissolved in 200 cc of carbon disulphide respectivelly and determined their own optical density (AKA type electric colorimeter. Cell; 1 cm,

Filter ; S_{50} for astaxanthin, S_{47} for yellow xanthophyll and carotenoid hydrocarbon). The results are summarized in Tables 4, 5 and Fig. 5.

Table 4. The optical density of astaxanthin, yellow xanthophyll and carotenoid hydrocarbon fraction.

Species	Optical density		
	Esterified xanthophyll		Carotenoid hydrocarbon
	Astaxanthin	Yellow xanthophyll	
Parabembras curtus	0.995	0.040	—*
Lepidotrigla microptera	0.680	0.135	—*
Sebastes inermis	1.250	0.260	0.040
Taius tumifrons	0.255	0.170	—*

* Carotenoid content was too minute to determine.

Table 5. Relation between the colour of skin and fins, and the ratio of optical density of astaxanthin to yellow xanthophyll.

Species	Colour of skin and fins	Optical density of astaxanthin / Optical density of yellow xanthophyll
Parabembras curtus	Red	24.9/1
Lepidotrigla microptera	Orange-red	5.0/1
Sebastes inermis	Dirty brown-red	4.8/1
Taius tumifrons	Yellow-pink	1.5/1

Summary

The distribution of carotenoids in the skin and fins of four species of marine fish has been examined. The amount of free xanthophyll and carotenoid hydrocarbon was minute and the esterified xanthophyll was dominant. Esterified xanthophyll was divided into two fractions ; a red xanthophyll which was recognized to be esterified astaxanthin and a yellow xanthophyll resembling sarcinaxanthin in the absorption maxima.

These two carotenoids were the principal pigment component of the skin and fins of each species. The relationship between the colour of the skin and fins of these fishes and the concentration of the two principal carotenoids was examined.

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EXPLANATION OF PLATE XXX

Fig. 1. Astacene from the skin and fins of *Parabembras curtus*.

Fig. 2. Astacene isolated from a crustacean decapod *Linuparus trigonus* VON SIEBOLD.

Fig. 3. Astacene from the skin and fins of *Lepidotrigla microptera*.

Fig. 4. Astacene from the skin and fins of *Sebastes inermis*.

Fig. 5. Astacene from the skin and fins of *Taius tumifrons*.

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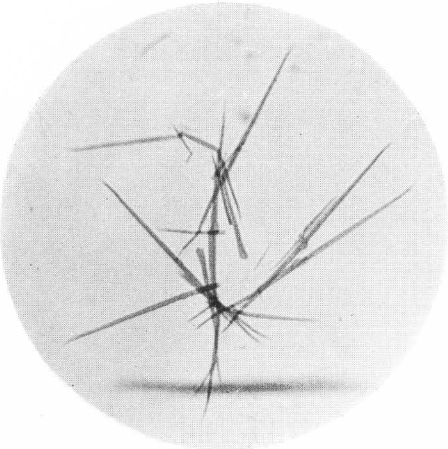

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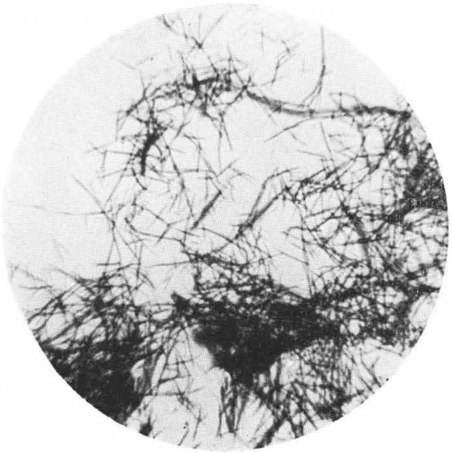

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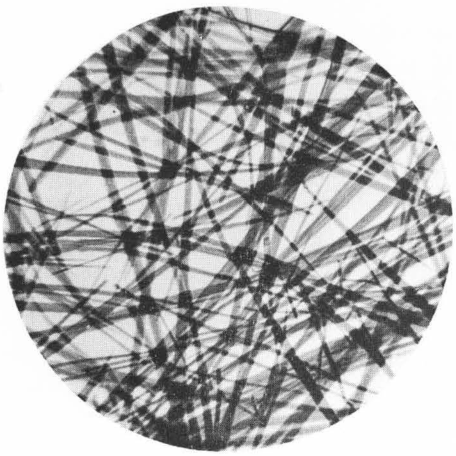

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